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# Carriage of *Clostridium difficile* by Wild Urban Norway Rats (*Rattus norvegicus*) and Black Rats (*Rattus rattus*)

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***Clostridium difficile* is an important cause of enteric infections in humans. Recently, concerns have been raised regarding whether animals could be a source of *C. difficile* spores. Although colonization has been identified in a number of domestic species, the ability of commensal pests to serve as a reservoir for *C. difficile* has not been well investigated. The objective of this study was to determine whether urban rats (*Rattus* spp.) from Vancouver, Canada, carry *C. difficile*. *Clostridium difficile* was isolated from the colon contents of trapped rats and was characterized using ribotyping, toxinotyping, and toxin gene identification. Generalized linear mixed models and spatial analysis were used to characterize the ecology of *C. difficile* in rats. *Clostridium difficile* was isolated from 95 of 724 (13.1%) rats, although prevalence differed from 0% to 46.7% among city blocks. The odds of being *C. difficile* positive decreased with increasing weight (odds ratio [OR], 0.67; 95% confidence interval [CI], 0.53 to 0.87), suggesting that carriage is more common in younger animals. The strains isolated included 9 ribotypes that matched recognized international designations, 5 identified by our laboratory in previous studies, and 21 "novel" ribotypes. Some strains were clustered geographically; however, the majority were dispersed throughout the study area, supporting environmental sources of exposure and widespread environmental contamination with a variety of *C. difficile* strains. Given that urban rats are the source of a number of other pathogens responsible for human morbidity and mortality, the potential for rats to be a source of *C. difficile* for humans deserves further consideration.**

*Clostridium difficile* is an obligately anaerobic bacterium and an important human pathogen (1, 2). It forms highly resistant spores, which can persist in the environment for long periods, facilitating transmission (3, 4). Clinical manifestations of *C. difficile* infection (CDI) in humans can range from asymptomatic carriage to mild diarrhea to fatal colitis (1, 4). Disease is a result of the proliferation of toxigenic strains of *C. difficile* and the production of toxins A and B (TcdA and TcdB), with some contribution from a third toxin, binary toxin (CDT) (5).

The ability of *C. difficile* to colonize the intestine is dependent on the disruption of the normal colonic microbiota (3, 6). For this reason, disease in humans is often precipitated by the administration of antibiotics (3, 6). Indeed, CDI is most common in hospitals and long-term-care facilities, particularly among the elderly and among those with comorbidities and receiving antibiotics (1, 3, 6).

Over the past few decades, there has been a marked increase in the incidence and severity of CDI (6). This is, in part, due to the emergence of "hypervirulent" and epidemic strains, particularly those of ribotypes 027 and 078, which are often more pathogenic, transmissible, and difficult to treat than other strains (1, 2, 6, 7). There has also been an increase in the incidence of community-associated CDI, which occurs in people with no history of hospitalization or antimicrobial therapy and in individuals previously thought to be at low risk (e.g., perinatal women) (1, 2, 6, 7).

A variety of animal species may also become colonized with *C. difficile* and/or may develop clinical disease secondary to infection (1, 4, 8). There is significant overlap of strains known to infect animals and humans, and in many cases, human and animal isolates are indistinguishable (1, 2, 4). This has raised the possibility of zoonotic transmission of *C. difficile* between animals and humans, either through direct contact, through the food chain (i.e., contamination of meat products), or through the environment (1, 6).

Although both companion and food-producing animals have been considered potential sources of *C. difficile*, the potential for commensal pests, particularly rodents, to be a reservoir for this bacterium has received little attention. A high prevalence of *C. difficile* carriage (66%), including carriage of ribotype 078, was detected in house mice (*Mus musculus*) infesting a pig farm in the Netherlands, where the researchers postulated that pests could play a role in the maintenance and transmission of *C. difficile* (9). Carriage of *C. difficile* in urban rodents, however, has not been investigated. Urban Norway rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) are known to be a significant source of disease for humans and can transmit a variety of different pathogens through direct contact, through contamination of food stuffs, or through the environment (10). This raises the question of whether urban rats could also be a source of *C. difficile*.

The objectives of this study were to determine if Norway and black rats from an inner-city neighborhood of Vancouver, Canada, carry *C. difficile*, to characterize isolated strains, and to begin to describe the ecology of *C. difficile* in urban rat populations.

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## MATERIALS AND METHODS

**Rat and tissue collection.** The study area comprised 43 city blocks (49°17′N, 123°6′W) within an inner-city neighborhood of Vancouver, Canada, as well as one property within the adjacent international shipping port terminal. Each block (and the port site) was randomly assigned to a 3-week study period over the course of 1 year (September 2011 to August 2012). Within each block, approximately 20 Tomahawk rigid traps for rats (Tomahawk Live Trap, Hazelhurst, WI, USA) were set out along each side of the back alley that bisected the block. At the port, traps were placed in areas where port staff had observed rats. Traps were prebaited (filled with bait but fixed open) for 1 week to acclimatize rats to trapping equipment and bait, followed by 2 weeks of active trapping. Trapped rats were anesthetized with isoflurane prior to pentobarbital euthanasia via intracardiac injection. Rats trapped at the port by a collaborating pest control professional using snap-type traps were also collected.

Morphometric data collected in the field included species (based on external morphology), sex, weight, sexual maturity (females with an open vaginal orifice and males with scrotal testes were considered sexually mature), and the presence and number of bite wounds in the skin (a proxy for social standing/interaction). The date and location (block and trap) of trapping was also recorded for each rat. Rats were subsequently frozen at −30°C and were sent to the Animal Health Centre (AHC), British Columbia Ministry of Agriculture, Abbotsford, British Columbia, Canada, for further analysis.

At the AHC, rats were thawed at 4°C and underwent a full necropsy between May and August 2012. During necropsy, the colon was collected aseptically and was stored at −80°C. Additionally, sex and sexual maturity were confirmed, and rats were examined for signs of pregnancy (visible embryos in the uterus) and lactation (well-developed mammary tissue and bare nipples). Each rat received a score based on the volume of internal fat stores (poor condition [score of zero], minimal to no visible internal fat; moderate condition [score of 1], moderate internal fat; good condition [score of 2], abundant internal fat).

This study was approved by the University of British Columbia's Animal Care Committee (A11-0087).

***Clostridium difficile* isolation, toxin gene identification, ribotyping, and toxinotyping.** Colonic contents were inoculated into 2 ml of *C. difficile* moxalactam norfloxacin (CDMN) enrichment broth (Oxoid Ltd., Nepean, Ontario, Canada) containing 0.1% sodium taurocholate. Samples were incubated anaerobically at 37°C for 7 days. A 1-ml aliquot of broth was mixed with an equal amount of anhydrous alcohol and was incubated at room temperature for 60 min. After centrifugation (at 3,800 × *g* for 10 min), the pellet was inoculated onto CDMN agar (Oxoid Ltd., Nepean, Ontario, Canada) and was incubated anaerobically at 37°C for 48 to 96 h. Suspicious colonies were subcultured onto Columbia blood agar and were incubated for 48 h at 37°C; subsequent identification was based on the characteristic morphology and odor of the colonies, Gram staining, and the presence of L-proline aminopeptidase activity (Remel Inc., Lenexa, Kansas, USA). A single colony for each isolate was subcultured, stored at −80°C, and recultured prior to molecular analysis.

All isolates identified as *C. difficile* were investigated for the presence of the toxin A (*tcdA*) (11), toxin B (*tcdB*) (12), and binary toxin (*cdtA*) (13) genes using PCR and were characterized using ribotyping (14). When a ribotype pattern was identified as an international ribotype, on the basis of comparison to the reference strains from the Cardiff-European Centre for Disease Prevention and Control (ECDC) collection, the appropriate numerical designation (e.g., 078) was assigned. Alternatively, an internal laboratory designation was assigned. Toxinotyping was also performed on all isolates (15).

**Statistical analysis.** The primary outcome variable was the presence of *C. difficile* (positive versus negative). Explanatory variables that were considered included species, the season of capture (September to November, fall; December to February, winter; March to May, spring; June to August, summer), weight, sex, sexual maturity (immature versus mature), fat score (score of 0 to 3), presence of cutaneous bite wounds, number of cutaneous bite wounds, and pregnancy and lactation in sexually mature females (see Table 2).

The distribution of each explanatory variable was examined among the samples as a whole, as well as separately for *C. difficile*-positive and -negative rats. Generalized linear mixed models (GLMM) were used to examine relationships between *C. difficile* positivity and each of the explanatory variables, first in a bivariate and then in a multivariate model, while controlling for clustering by city block of origin. The goal for the final multivariate GLMM was to identify the most parsimonious set of explanatory variables that predicted the outcome.

To determine whether the epidemiology of *C. difficile* differed by ribotype, the multivariate-model-building process was repeated for rats with "novel strains" (i.e., those not consistent with an international designation and not previously identified in our laboratory) versus *C. difficile*-negative rats (rats with previously identified strains were excluded), and for rats with previously identified strains versus *C. difficile*-negative (rats with "novel" strains were excluded).

Finally, the city block was entered into a simple logistic regression model as a fixed effect in order to determine whether the block of origin was significantly associated with the odds of an individual rat being infected with *C. difficile*.

All statistical analyses were conducted using R (R Development Core Team, Vienna, Austria). For multivariable models, individuals with missing data for one or more of the variables under study were excluded.

**Spatial analysis.** The location of each trap within the study area, the number of rats caught in each trap that were tested for *C. difficile*, and the number of rats caught in each trap that were *C. difficile* positive were mapped using ArcGIS, version 10.0 (Esri, Redlands, CA, USA). For *C. difficile*-positive rats, the ribotype that each rat was carrying was also mapped. This information was imported into SaTScan, version 9.1.1 (Martin Kulldorff/Information Management Services Inc., Boston, MA, USA), for cluster analysis using a purely spatial Bernoulli model, and scanning for areas with high and low rates of *C. difficile* carriage was carried out using a circular window with a maximum spatial cluster size of 50% of the population at risk. SaTScan uses a scanning window statistic to identify the clustering of observed events ("cases") compared with the distribution of events that would be expected ("controls") if the spatial locations of all events were independent.

For the first analysis, any *C. difficile*-positive rat was considered a "case" and any *C. difficile*-negative rat was considered a "control." Subsequently, this analysis was repeated separately for each ribotype with more than one isolate in order to identify any areas of clustering by ribotype. For these analyses, any rat with the ribotype of interest was considered a "case" and all *C. difficile*-positive rats with other ribotypes were considered "controls."

For all spatial analyses, the port site was excluded because of privacy concerns (trapping occurred in private property), because trapping took place at multiple levels within a single geographic footprint (which is difficult to represent in a 2-dimensional map), and because trapping was somewhat more opportunistic (versus systematic) than in the blocks.

## RESULTS

*Clostridium difficile* was detected in 95 of 724 (13.1%) rats trapped, although the prevalence differed markedly by city block, from 0% to 46.7% (Fig. 1). The block of origin was significantly associated with the odds of being *C. difficile* positive (data not shown).

Of the 95 isolates, 1 (1.1%) had *tcdB* but not *tcdA* or *cdtA*; 78 (82%) had *tcdA* and *tcdB* but not *cdtA*; and 16 (16.9%) had all three toxin genes. A total of 35 different ribotypes were identified (Table 1). These included 9 ribotypes that matched recognized international designations, 5 ribotypes identified by our laboratory in human samples from previous studies but not matching international designations, and 21 ribotypes not previously iden-

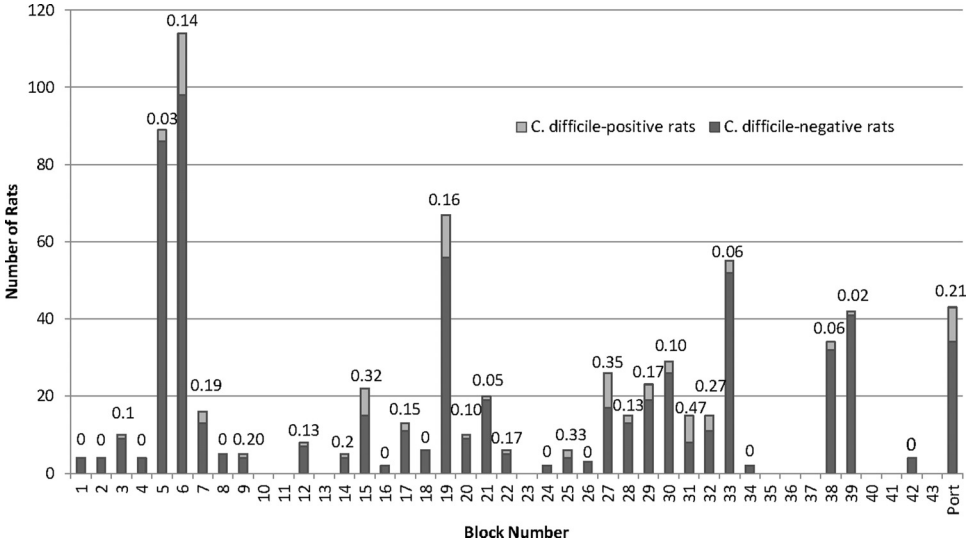


**FIG 1** Numbers of *Clostridium difficile*-positive and *Clostridium difficile*-negative rats in each city block and at the port site. The prevalence of *C. difficile* is noted above each bar.

tified by our laboratory in humans or animals (and not matching international designations).

There was no evidence of geographic clustering for *C. difficile*-positive rats in general (Fig. 2A). However, clusters were identified for ribotypes 078, A, and VR6 (Fig. 2B and C). The ribotypes identified at the port included 001 ($n = 7$), 014 ($n = 1$), and VR11 ($n = 1$).

The distribution of the explanatory variables in the sample as a whole, and in *C. difficile*-positive and -negative rats, is detailed in Table 2. After controlling for clustering by block, only weight was a significant predictor of *C. difficile* status in either bivariate or multivariate models (Table 3); a weight increase of 10 g was associated with 33%-decreased odds of being *C. difficile* positive (odds ratio [OR], 0.67; 95% confidence interval [CI], 0.53 to 0.87). In this model, the variance associated with the random effect of the block of origin was 0.45. The final model did not change significantly when "novel" or previously recognized ribotypes were used as the outcome of interest (data not presented).

## DISCUSSION

Canada is one of the many countries experiencing a significant and growing health burden associated with CDI. Indeed, within the province of British Columbia (BC), where Vancouver is situated, *C. difficile* has overtaken methicillin-resistant *Staphylococcus aureus* as the most common cause of health care-associated (HCA) infection in acute-care facilities (16). In 2012 to 2013, 3,246 cases of CDI were reported in BC acute-care facilities; of these, 72.6% were classified as HCA and 27.4% were classified as community associated (CA) or of unknown association (16). Within the Vancouver Coastal Health Authority, specifically, the annual incidence rate of CDI in 2012 to 2013 was 8.4 cases per 10,000 inpatient days (16). Of particular concern was a 69.8% increase in the number of CA CDI cases in BC from 2009–2010 to 2012–2013 (16). The source of *C. difficile* in cases of CA CDI is not well understood but could potentially include strains of animal origin.

To our knowledge, this is the first study to demonstrate that wild urban Norway and black rats can carry *C. difficile*. The ribotypes identified for these rats included 9 internationally recognized ribotypes known to colonize and/or cause disease in humans and domestic animals (17, 18), 5 ribotypes without international designations but previously identified in human samples at our laboratory, and 21 ribotypes without international designations and not previously identified by our laboratory among a collection of >5,000 isolates from humans and animals.

Ribotype 001, a North American pulsotype 2 (NAP2) strain, is an important cause of CDI in humans in Canada (19) and was one of the most common ribotypes identified in this study. This ribotype has also been identified in various animal species in Canada, including dogs, cats, and horses (20, 21). Ribotype 078, which is an emerging cause of CA CDI in humans (7), was also relatively common among the rats studied here. This ribotype is frequently isolated from livestock (22, 23), leading to concerns that zoonotic transmission may be a significant source of this pathogen for humans (7, 24). While only one rat harbored ribotype 027/NAP1, this is noteworthy because of its clinical importance in humans (2, 6). This ribotype, which belongs to toxinotype III and possesses CDT, is considered to be an epidemic hypervirulent strain, since it accounts for a significant proportion of CDI outbreaks in humans and is associated with increased disease severity and an increased rate of relapse (25, 26). One novel strain detected in 5 rats in this study, VR8, also belonged to toxinotype III and was CDT positive. Whether this strain poses the same human health risks as ribotype 027 is unknown but should be considered. Ribotype 014, found in 3 rats in this study, is another common pathogenic strain in humans and has been found in various animal species (27, 28). It was reported to be the most common ribotype in a study of river water in Slovenia (29), which could suggest that water is a source of exposure for rats and/or that rats are a source of contamination of water with this strain.

Twenty-one "novel" ribotypes were identified in this study and accounted for 52% of isolates. Lack of harmonization of ribotyping methodology and the absence of a comprehensive comparative database hinder accurate epidemiologic analysis of *C. difficile*

**TABLE 1** Relative frequencies of *Clostridium difficile* ribotypes (with toxin profile and toxinotype) isolated from urban Norway rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) in Vancouver, Canada

| Ribotype[a] | Toxin gene(s) | Toxinotype | No. (%) of isolates |
|---|---|---|---|
| 001 | *tcdA*, *tcdB* | 0 | 12 (1.7) |
| 002 | *tcdA*, *tcdB* | 0 | 3 (0.4) |
| 005 | *tcdA*, *tcdB* | 0 | 1 (0.1) |
| 012 | *tcdA*, *tcdB* | 0 | 1 (0.1) |
| 014 | *tcdA*, *tcdB* | 0 | 3 (0.4) |
| 017 | *tcdB* | VIII | 1 (0.1) |
| 027 | *tcdA*, *tcdB*, *cdtA* | III | 1 (0.1) |
| 078 | *tcdA*, *tcdB*, *cdtA* | V | 8 (1.1) |
| 137 | *tcdA*, *tcdB* | 0 | 1 (0.1) |
| A | *tcdA*, *tcdB* | XIII | 7 (1.0) |
| AJ | *tcdA*, *tcdB* | XII | 1 (0.1) |
| F | *tcdA*, *tcdB* | 0 | 2 (0.3) |
| O | *tcdA*, *tcdB* | 0 | 4 (0.6) |
| Q | *tcdA*, *tcdB* | XII | 1 (0.1) |
| VR1 | *tcdA*, *tcdB* | 0 | 1 (0.1) |
| VR2 | *tcdA*, *tcdB* | 0 | 12 (1.7) |
| VR3 | *tcdA*, *tcdB* | I | 1 (0.1) |
| VR4 | *tcdA*, *tcdB* | XII | 1 (0.1) |
| VR5 | *tcdA*, *tcdB* | XII | 1 (0.1) |
| VR6 | *tcdA*, *tcdB* | 0 | 2 (0.3) |
| VR7 | *tcdA*, *tcdB* | XII | 4 (0.6) |
| VR8 | *tcdA*, *tcdB*, *cdtA* | III | 5 (0.7) |
| VR9 | *tcdA*, *tcdB* | 0 | 1 (0.1) |
| VR10 | *tcdA*, *tcdB*, *cdtA* | IV | 1 (0.1) |
| VR11 | *tcdA*, *tcdB* | 0 | 3 (0.4) |
| VR12 | *tcdA*, *tcdB* | 0 | 1 (0.1) |
| VR13 | *tcdA*, *tcdB* | 0 | 1 (0.1) |
| VR14 | *tcdA*, *tcdB* | 0 | 4 (0.6) |
| VR15 | *tcdA*, *tcdB* | XII | 1 (0.1) |
| VR16 | *tcdA*, *tcdB* | XXI | 2 (0.3) |
| VR17 | *tcdA*, *tcdB* | 0 | 1 (0.1) |
| VR18 | *tcdA*, *tcdB* | XXI | 4 (0.6) |
| VR19 | *tcdA*, *tcdB* | XII | 1 (0.1) |
| VR20 | *tcdA*, *tcdB* | 0 | 1 (0.1) |
| VR21 | *tcdA*, *tcdB*, *cdtA* | V | 1 (0.1) |

[a] Numerical identifiers (e.g., 001) match international designations. Letter identifiers (e.g., A) were assigned to ribotypes not matching international designations but previously identified in our laboratory. VR designations (e.g., VR1) were assigned to ribotypes not matching international designations and not previously identified in our laboratory.

strains; therefore, whether these ribotypes have ever been identified in humans or other species cannot be definitively determined. However, the fact that these strains have not been found in our large and diverse isolate collection suggests that at least some might be associated with rats or wildlife, or at least might be rare in other species. Ultimately, the risk posed to humans or other animal species by these "novel" strains is unclear at this time.

The only factor significantly associated with *C. difficile* infection in these rats was weight. Increased body weight was significantly associated with decreased odds of being *C. difficile* positive, suggesting that carriage is more common in younger rats. Indeed the median weight for *C. difficile*-positive rats was 90.6 g versus 147.7 g for C. *difficile*-negative rats. Weight is often used as a proxy for chronologic age in rats but can also be influenced by a variety of other factors, including sex, nutritional condition, species, and even population of origin (30–34). In this study, species, sex, internal fat stores, and block of origin were controlled for in the analysis, leading us to believe that the association between *C. difficile* and weight likely suggests that carriage is, indeed, a function of age. This pattern is similar to that for humans and other animal

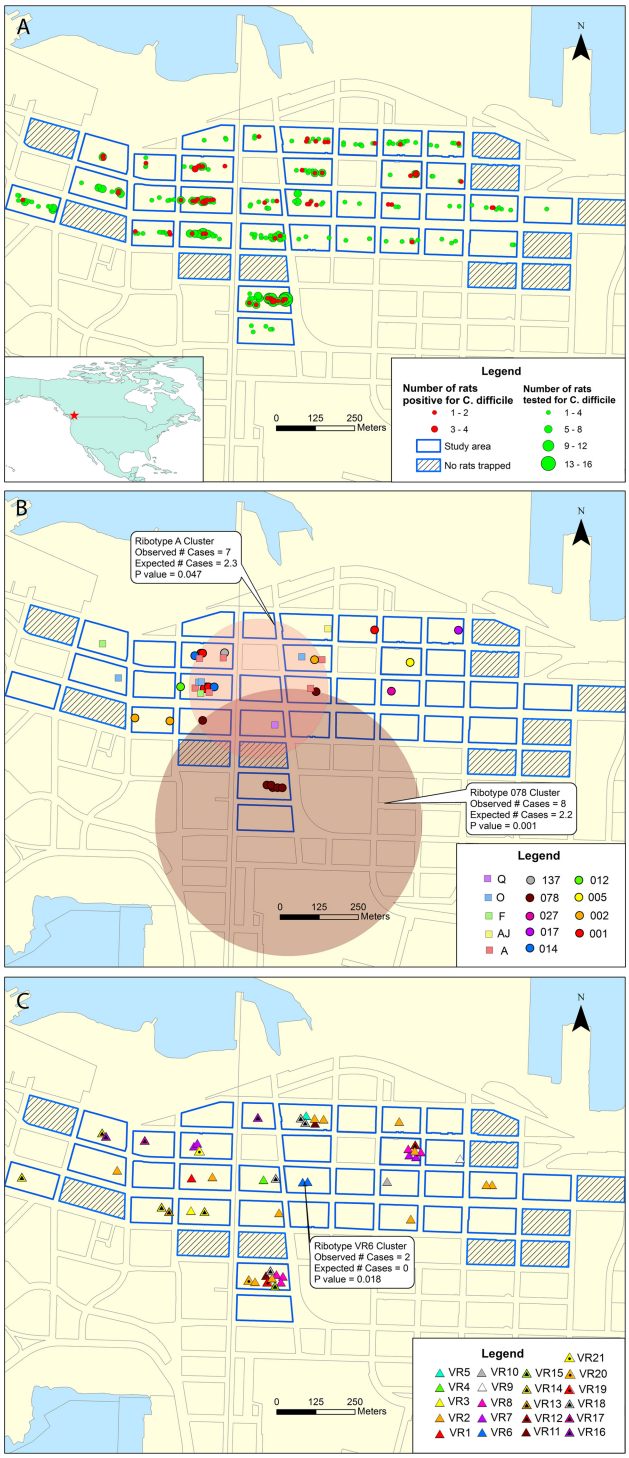


**FIG 2** (A) Geographic distribution of *Clostridium difficile*-positive rats. (B) Distribution of *C. difficile* ribotypes with international designations or previously identified in our laboratory and clusters of high prevalence. (C) Distribution of "novel" *C. difficile* ribotypes and a cluster of high prevalence.

**TABLE 2** Baseline characteristics and associations with *Clostridium difficile* status among urban Norway rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) from Vancouver, Canada

| Category[a] | No. (%)[b] of rats: Total ($n$ = 724) | Positive for *C. difficile* ($n$ = 95) | Negative for *C. difficile* ($n$ = 629) | $P$[c] |
|---|---|---|---|---|
| Species | | | | |
| Norway | 684 (94.5) | 89 (93.7) | 595 (94.6) | 0.90 |
| Black | 40 (5.5) | 6 (6.3) | 34 (5.4) | |
| Season | | | | |
| Fall | 239 (33.0) | 23 (24.2) | 216 (34.3) | <0.01 |
| Winter | 135 (18.6) | 23 (24.2) | 112 (17.8) | |
| Spring | 259 (35.8) | 45 (47.4) | 214 (34.0) | |
| Summer | 91 (12.6) | 4 (4.2) | 87(13.8) | |
| Sex | | | | |
| Male | 400 (55.2) | 48 (50.5) | 352 (56.0) | 0.51 |
| Female | 316 (43.6) | 44 (46.3) | 272 (43.2) | |
| Sexual maturity | | | | |
| Mature | 417 (57.6) | 47 (49.5) | 370 (58.8) | 0.06 |
| Immature | 237 (32.7) | 40 (42.1) | 197 (31.3) | |
| Wt (g) (median [IQR]) | 134.0 (63.8–251.6) | 90.6 (56.7–215.2) | 147.7 (65.8–257.6) | <0.01 |
| Fat score | | | | |
| Poor | 307 (42.4) | 47 (49.5) | 260 (41.3) | 0.15 |
| Moderate | 195 (26.9) | 25 (26.3) | 170 (27.0) | |
| Good | 202 (27.9) | 19 (20.0) | 183 (29.1) | |
| Wound presence | | | | |
| Yes | 176 (24.3) | 20 (21.1) | 156 (24.8) | 0.50 |
| No | 547 (75.6) | 75 (78.9) | 472 (75.0) | |
| Median no. of wounds (IQR) | 0.0 (0.0–0.0) | 0.0 (0.0–0.0) | 0.0 (0.0–0.0) | 0.30 |
| Pregnant | | | | |
| Yes | 33 (20.0) | 2 (7.4) | 31 (22.5) | 0.12 |
| No | 132 (80.0) | 25 (92.6) | 107 (77.5) | |
| Lactating | | | | |
| Yes | 79 (47.9) | 12 (44.4) | 67 (48.6) | 0.86 |
| No | 86 (52.1) | 15 (55.6) | 71 (51.4) | |

[a] IQR, interquartile range. Pregnancy or lactation was determined for sexually mature females only ($n$ = 165).
[b] Except where other measurements are indicated. Frequencies and percentages may not add up to 100% because of the exclusion of rats with missing data for the variable in question.
[c] Determined using the chi-square test or Welch's $t$ test, where appropriate.

species, where colonization is most common in the young (23, 35), likely because an immature gut microbiota is conducive to the establishment of *C. difficile* (36). It should be noted that the rats included in this study, by virtue of being in the "trappable" population, were weaned and had left the nest (37). It would be interesting to know whether the prevalence of *C. difficile* is even greater in unweaned rats, as it is in infant humans (35, 38, 39).

Although within the study site as a whole there was no clear clustering of *C. difficile*-positive rats, the prevalence of *C. difficile* did differ significantly by city block (which was also reflected by the variance associated with the random effect of block in the GLMM). This suggests that while no one area was particularly conducive to *C. difficile* carriage, there may be block-level differences in environmental or population characteristics that impact the probability of *C. difficile* carriage in rats. Similarly, the majority of the ribotypes appeared to be geographically dispersed. Since urban rats exist in tight-knit colonies with small home ranges (usually limited to a city block) and minimal intercolony contact (33, 40), the overall paucity of clustering by ribotype seems to suggest that transmission of *C. difficile* among rats is minimal. Rather, it appears more likely that rats acquire *C. difficile* from the environment and that environmental contamination with numerous different *C. difficile* ribotypes is ubiquitous within the study area. Environmental exposure is thought to be among the most important routes of *C. difficile* infection for humans and other species (1, 3, 35). Significant geographic clusters of ribotypes 078, A, and VR6 (and likely ribotype 001 at the port) might suggest that some transmission among rats is possible but might also indicate a common environmental source of exposure.

The exact source of exposure, however, is difficult to determine. Within the urban environment, rats have the potential to come into contact with spores under a number of different cir-

**TABLE 3** Relationship between rat characteristics and *Clostridium difficile* status among Norway rats (*Rattus norvegicus*) and black rats (*Rattus rattus*) from Vancouver, Canada

| Category[a] | OR (95% CI)[b] | |
|---|---|---|
| | Unadjusted[c] | Adjusted[d] |
| Species | | |
| Black | 2.05 (0.57–7.34) | |
| Norway | REF | |
| Season | | |
| Winter | 1.64 (0.71–3.82) | — |
| Spring | 1.83 (0.84–3.96) | — |
| Summer | 0.45 (0.12–1.74) | — |
| Fall | REF | |
| Sex | | |
| Male | 0.86 (0.55–1.36) | — |
| Female | REF | |
| Maturity | | |
| Immature | 1.59 (0.95–2.66) | — |
| Mature | REF | |
| Wound presence | | |
| Yes | 0.69 (0.40–1.20) | — |
| No | REF | |
| No. of wounds | 0.88 (0.71–1.08) | — |
| Wt (per 10 g) | 0.67 (0.53–0.87) | 0.67 (0.53–0.87) |
| Fat score (categorical) | | |
| Good | 0.55 (0.28–1.04) | — |
| Moderate | 0.84 (0.47–1.48) | — |
| Poor | REF | |
| Pregnant | | |
| Yes | 0.30 (0.06–1.41) | — |
| No | REF | |
| Lactating | | |
| Yes | 0.95 (0.39–2.35) | — |
| No | REF | |

[a] Values for pregnancy and lactation were determined for sexually mature females only ($n = 165$).
[b] Generated by using a generalized linear mixed model to control for clustering by city block of origin. REF, reference category.
[c] Results of bivariate modeling.
[d] Results of the final multivariate-model-building procedure. —, variable not included in the final model.

cumstances, for example, while foraging in refuse or even while simply traversing the alley surface. It should be noted that there is a significant amount of human excrement in the alleys within our study area, which could serve as a source of exposure for rats. Rats might also be exposed to spores in sewage, although it cannot be determined if the rats included in this study access the sewer systems. Within the study area, there are a number of facilities that prepare and sell animal products and are thus a potential source of strains of livestock origin. A number of companion animals, such as cats and dogs, also reside in the neighborhood. Finally, it is also possible that rats might become exposed to spores in the feces of other rats either through contamination of the burrow system or through coprophagic behaviors in rat pups (41, 42).

It is noteworthy that there was no clinical or postmortem evidence of diarrhea or enteritis in any of the *C. difficile*-positive rats. This is consistent with the literature, which suggests that rats are relatively refractory to CDI (8). However, all of the strains identified carried at least one toxin gene; 82% carried both the *tcdA* and *tcdB* genes; and 16.9% carried all three genes. Additionally, although most ($n = 28$) *C. difficile* strains identified in this study belonged to toxinotype 0, five other toxinotypes were found, including toxinotypes III and V, which receive much attention because they include ribotypes 027 and 078, respectively (15). This suggests that the *C. difficile* strains carried by these rats could be pathogenic for humans and other species (1, 3, 8). The potential for rats to be a significant source of *C. difficile* is amplified by their capacity to contaminate the environment, particularly foodstuffs, with their feces (33). Indeed, fecal contamination of food is a known route of exposure to other rat-associated zoonoses (10), and there is evidence that zoonotic or food-borne transmission may be an important component of the epidemiology of certain *C. difficile* strains, such as ribotype 078 (43).

One limitation of the current study was its cross-sectional nature, which prevented us from being able to determine to what degree *C. difficile* positivity represents true colonization versus transient passage of spores. Future studies ought to include longitudinal sampling, if possible, in order to determine the degree to which *C. difficile* can be maintained and propagated in rat populations. Additionally, we chose to characterize only one *C. difficile* isolate per rat and therefore could not address the possibility that a single rat could carry more than one ribotype. It may be worthwhile to characterize multiple isolates per individual in the future in order to better characterize the ecology of *C. difficile* in rats.

Past research on rat-associated zoonotic risks has focused largely on pathogens for which the rat is the natural host (e.g., *Leptospira interrogans*, *Streptobacillus moniliformis*, and *Yersinia pestis*). However, this study shows that urban rats can become colonized with other pathogens present in their environment and could subsequently serve as a reservoir for these organisms. Given the exploratory nature of rats (44) and their propensity to inhabit and/or exploit every aspect of the urban ecosystem (33), the capacity for rats to accumulate pathogenic microbes should not be underestimated. Overall, the frequency and diversity of *C. difficile* strains identified in this study, including novel strains as well as those commonly found in humans and domestic animals, suggest that the ecology of *C. difficile* in urban rats is complex and warrants further study.

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